Methods: Confluent MSCs from 6 OA patients and 6 controls were trypsinized, counted, and then washed once by centrifugation. Chemotaxis responses were measured using 24-well cell-culture chambers (Transwell® Permeable Supports; Corning, NY), with inserts of 6.5-mm-diameter and 8-μm pore polycarbonate membranes. Cells (2×10^4) in 0.1 ml DMEM were seeded on the upper chambers and incubated for 2 hour at 37 ºC in 5% CO_2 to allow cell attachment and spreading. After the cells had attached to the Transwell® membranes, 0.6 ml culture medium containing 1 ng/ml rhPDGF-BB (R&D Systems Inc.) was added to the lower compartment. Following 4 h of incubation at the same temperature, the cells on the upper surface of the membrane were removed with a cotton swab, and the remaining migrated cells on the lower side of the filter were fixed with 4% formaldehyde and stained with crystal violet. Control wells with only DMEM in the bottom well were included in each experiment. All measurements were from at least six independent experiments performed in triplicate.
The number of migrated cells in the control and stimulated wells was counted for 10 random fields per well at 100× magnification. Results were expressed as a chemotactic index (CI). This index was determined as the average number of migrated cells in stimulated wells divided by the average number of migrated cells in control wells. Data were statistically analysed with the Mann-Whitney U test for comparison of the two groups. Results are presented as means±standard deviation. A value of $P < 0.05$ was considered statistically significant.
Results: rhPDGF-BB (1 ng/ml) added to the bottom compartment of the Transwell insert caused a significant increase in migrating MSCs from patients with OA compared to controls (CI: 5.13±1.19 *vs.* 3.35±0.42, $p = 0.043$). When identical amounts of rhPDGF-BB were simultaneously added to the both the upper and the lower compartments, the cell migration of MSC remained unchanged.
Conclusions: These data could indicate an activation of MSCs from bone marrow in OA in response to permanent signals sent by the bone and cartilage damage characteristic of this disease.

486 THE PRESENCE OF A CALCIFIED INTERFACE STRENGTHENS TISSUE ENGINEERED CARTILAGE-SUBSTRATE INTERFACIAL SHEAR STRENGTH

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Purpose: To design tissue engineered cartilage with improved attachment to a porous calcium polyphosphate (CPP) bone substitute (biphasic construct) by the formation of a zone of calcified cartilage (ZCC) at the cartilage-CPP interface zone.
Methods: Cartilage-CPP biphasic implants were formed by seeding chondrocytes isolated from the deep-zone of bovine articular cartilage onto the top surface of porous CPP substrates coated with a thin sol-gel-formed hydroxyapatite film. The cells were grown in Ham's F12 containing 20% FBS in the absence or presence of β-glycerophosphate supplementation to induce mineralization. After 8 weeks, tissues were harvested and examined histologically, biochemically, by RT-PCR for gene expression and for the shear properties of the interface between cartilage and the substrate using a specifically designed shear test apparatus.
Results: Histological sections stained with toluidine blue and von Kossa demonstrated cartilage tissue with a thin ZCC when cultured in mineralization-inducing media. The ZCC was localized in the tissue directly above the substrate, mimicking osteochondral zonal organization. The mineral formed in cartilage *in vitro* under these conditions has similar composition and crystal size to that present in the ZCC of native cartilage. Conversely, no calcification was observed in cartilage formed in the absence of β-glycerophosphate. Collagen and glycosaminoglycan contents were similar for tissues with and without a calcified zone. Expression levels of cartilage extracellular matrix genes including collagen type II and type X were not affected by the generation of a ZCC. Interestingly, the shear stiffness of the interface was increased by 2-fold in the tissue engineered cartilage with a ZCC compared to cartilage without a ZZC. This was associated with 240% improvement in the peak shear load and in 340% increase in energy to failure measurements. The maximal displacement prior to failure was similar in both tissues.
Conclusions: This study demonstrates a significant increase in interfacial shear properties of biphasic cartilage constructs by mimicking the zonal organization of articular cartilage by generating a ZCC at the tissue/bone substitute interface. Generating biphasic cartilage constructs with a calcified cartilage interface will be critical for the clinical success of biphasic constructs used to repair larger joint defects.

487 THE EFFECT OF LOW-INTENSITY PULSED ULTRASOUND FOR FORMING OF SCAFFOLD-FREE CARTILAGE TISSUE IN VITRO

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Purpose: The aim of this study is to evaluate the effect of low-intensity pulsed ultrasound (LIPUS) for forming of scaffold-free cartilage in vitro culture.
Methods: Chondrocytes were collected from articular cartilage of Wistar rats. For acquirement the number of cells, the collected primary chondrocytes (passage 0) were cultured up to subconfluent in 500 cm^2 square dishes. The cells were then condensed to the density at 10^7 cells/cm^2 (passage 1) on synthetic membranes. The LIPUS application group was stimulated by LIPUS for 20 min/day. The mode of the applied ultrasound is a 200 μs burst sine wave of 1.5 MHz repeating at 1 kHz with an intensity of 30 mW/cm^2. To investigate effect LIPUS stimulation on the matrix-synthesis of the tissues, mRNA expression of type II collagen (col2), aggrecan and type I collagen (col1) was studied using real-time polymerase chain reaction. Synthesis of type II collagen and proteoglycan was also assessed histochemically in 4weeks culture.
Results: In our previous study, we presented that high-density culture with P1 chondrocytes more than 10^6 cells/cm^2 could form a cell mass as scaffold-free cartilage tissue under existing cell-cell interactions.
In this way, the chondrocytes prepared at 10^7 cells/cm^2 automatically detached from the membranes to form a mass of chondrocytes around the third day of starting P1 culture. After forming the mass, they were moved to simple 6 well dishes for oxygen and nutrition diffusion. The expression of col2 and aggrecan mRNA was significantly higher in the group by stimulation of LIPUS than the group by no stimulation.
The resultant scaffold-free cartilage like tissues formed via cell condensation at 10^7cells/cm^2 grew in thickness until the 7th day of P1 culture. They grew in size until the 14th day of P1 culture.
The tissues were stained strongly with safranin O, and there were partially columnar pattern. Immunohistochemical analysis revealed that typeIIcollagen was abundantly deposited in the tissues. Moreover with toluidine blue staining, there was a metachromatic matrix in them. Respectively, LIPUS stimulation group was more strongly stained than the control group.
Conclusions: The chondrocytes of P1 cells prepared at 10^7 cells/cm^2 developed into scaffold-free cartilage like tissue under existing cell-cell interactions. As a result of application of LIPUS, we could form scaffold-free cartilage like tissues which were similar to native cartilage.

488 BONE-MARROW-DERIVED CELLS (BMDCS) ONE STEP REPAIR PROCEDURE ("ONE STEP") VERSUS AUTOLOGOUS CHONDROCYTE IMPLANTATION (ACI) IN SURGICAL TREATMENT OF OSTEOCHONDRAL LESIONS OF THE TALUS: A COMPARATIVE HISTOLOGICAL STUDY

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Purpose: Osteochondral lesions of the talus are frequent occurrences in young active patients. Various surgical options have been proposed over time in order to restore a continuous and adequate cartilaginous layer at the lesion site. Among them, only mosaicplasty and Autologous Chondrocyte Implantation (ACI) were capable, up to now, to restore hyaline cartilage at the lesion site, although major disadvantages of these techniques have been widely described. These considerations leaded in search of new methods for cartilage repair and recently the use of Bone Marrow-Derived Cells (BMDCs) was indicated as a reliable alternative for the treatment of articular cartilage defects. The aim of this study was to verify the ability of an original technique based upon BMDCs to regenerate hyaline articular cartilage, and to compare the clinical and histological results with them obtained after ACI.
Methods: From October 2005 to April 2008, 50 patients affected by osteochondral lesions of the talus >1.5 cm^2 underwent a new arthroscopic "One Step" procedure by using bone marrow harvested from the iliac crest, directly concentrated in Operatory Room and seeded on a scaffold with the addition of Platelet-Rich-Fibrin (PRF).
Patients evaluation included clinical AOFAS score, X-Rays, MRI preoperatively and at different established follow-up. A series of 46 patients operated by ACI procedure and comparable for age and lesion types was used as control group. At 12 months follow up after "One Step" procedure, an arthroscopic second look and a biopsy of the regenerated cartilage